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THE EFFECT ON THE OFFSPRING OF INTOXICATING THE MALE PARENT AND THE TRANSMISSION OF THE DEFECTS TO SUBSEQUENT GENERATIONS

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It is a thoroughly demonstrated fact that the fertilized egg may be so treated or modified during development as to cause it to give rise to abnormal embryos of definite types. Experiments on the unfertilized egg or female germ cell are not nearly so numerous, are more difficult to perform, and the results are not so decided. The treatment of the male germ cell, or spermatozoon, so as to modify it and to cause a modified development of the egg which it subsequently fertilizes, is an experiment which has rarely been performed with success. In the present communication we wish to consider rather briefly the various methods of treating or modifying the spermatozoon or male germ cell and the result of this modification on the embryo which arises when such a spermatozoon fertilizes an egg. In order to fully appreciate the results obtained by experiments on the sperm it becomes necessary to refer from time to time to the effects derived when the egg is similarly treated.

Since the positive literature bearing on the artificial modification of the sperm is not extensive, I shall first consider it in a general way, and devote the latter part of the paper to the results obtained in a set of experiments

which have been conducted on guinea-pigs for the last few years.

The spermatozoon is more difficult to treat experimentally than is the ovum, on account of the fact that the treatment so often renders it inactive or cripples it in such a way that it is unable to penetrate and to fertilize an egg. The spermatozoon, although apparently delicate, is more or less resistant, so that a mild treatment gives no effect. The difference between the upper non-effective dose of treatment and the fatal or "paralyzing" dose is slight, yet it is the precise treatment between these two points which yields results.

Little has been done in treating chemically the spermatozoa of invertebrates, though some of the hybridization experiments furnish indirect evidence as to what might occur. Herbst's experiment of starting the development of an egg by parthenogenesis and then fertilizing one blastomere with a foreign spermatozoon offers splendid opportunity for investigating the influence of strange substances introduced by the sperm. The spermatozoa of the sea urchin have been subjected to the action of radium emanations by Günther Hertwig, who found that after intensive treatment for several hours such spermatozoa subsequently disturb the processes of division in the eggs which they have fertilized. Paula Hertwig found that fertilized ascaris eggs treated several hours with radium preparations gave pathological division figures; the chromatin bodies showed a tendency to break down and disintegrate; division was slow and the karyokinetic figures were finally completely deranged.

Investigations on vertebrates have been more extensive. The most beautiful results have been obtained on fish and amphibians through the radium experiments of Oscar Hertwig, his son Günther and his daughter Paula. The outcome of these experiments is so striking and is of such serious importance that I shall dwell somewhat upon their significance. About three years ago O. Hertwig published the results of his first radium experiments in

the *Proceedings of the Prussian Academy of Sciences*. At that time he showed that when the unfertilized eggs of a frog are treated for a certain time with radium rays, these eggs, after being fertilized by normal spermatozoa, develop abnormally. Hertwig also found that when the milt or semen of the frog was exposed to the action of radium for a certain time the spermatozoa swimming in it were injured. When eggs were fertilized by these sperm they always developed abnormally. The general type of the abnormalities was the same whether the eggs alone had been treated before fertilization or whether the sperm was treated. When both eggs and sperm were treated the developmental modifications were still more pronounced, though Hertwig claims that the deformities were of a similar type to those which occurred after treating only one of the cells.

Since that time Oscar Hertwig with his son and daughter have extended and analyzed these experiments in a comprehensive fashion. When the sperm of a number of amphibians, frog, toad and salamander, are exposed for five minutes to 5.3 mg. of radium bromide, normal eggs fertilized by such sperm give defective embryos, the defects being generally shown by the central nervous system. They are really of the nature of developmental arrests or degeneration.

If the spermatozoa are exposed for fifteen minutes the effects on development are still more marked. When, however, the sperm in salt solutions are treated intensively for 2 and 3 hours between two mesothorium capsules, the results are most surprising. In one experiment almost all the eggs fertilized by such sperm went *normally*, and in other experiments they went almost normal but slow, yet they were extraordinarily better than eggs that were fertilized by sperm that had been treated for only five minutes. After three weeks the radium larvæ were still behind the control. Hertwig concluded that the spermatozoon had been so injured by the intensive treatment that it could no longer take part in development,

although it could penetrate the egg and cause it to develop parthenogenetically.

A number of important experiments were tried to test the correctness of this conclusion. The most striking of these were those performed by Günther Hertwig in crossing different species.

It is well known that the sperm of the frog will fertilize the egg of the toad or of another species of frog, but the resulting development proceeds for only a short time and the egg usually dies in the blastulæ stage. Günther Hertwig decided that if the influence of the sperm in development was really destroyed by the intensive radium treatments then such a spermatozoon would merely serve as a parthenogenetic agent and the egg should develop in a normal manner, yet be parthenogenetic. He used the eggs of *Bufo vulgaris* (the common toad) and of *Rana veridis* (the green water frog), and the sperm of *Rana fusca*. He ran two sets of each kind of eggs; one set was fertilized by normal *R. fusca* sperm; the other by sperm which had been treated for two or three hours between two capsules of mesothorium. The eggs fertilized by normal sperm did not develop beyond the blastular stage as was expected, while those fertilized with the radiumized sperm developed about normally and hatched from the jelly and gave rise to swimming tadpoles. O. Hertwig repeated this experiment by crossing the eggs of *Triton vulgaris* with the sperm of *Salamandra maculata*. The sperm were treated for $2\frac{1}{4}$ hours between two strong mesothorium preparations. Poll had found that this cross proceeded only as far as the blastular stage and Hertwig confirmed this.

When, however, the semen was treated for $2\frac{1}{4}$ hours a different result was obtained. Many eggs failed to become fertilized, some showed polyspermy, and only six went normally. The chromatin of the sperm was thought to be destroyed and the eggs went by parthenogenesis. Loeb has found in the remarkably wide crosses he has made on invertebrates and vertebrates that the products

of part of the foreign sperm finally act as a poison and cause the eggs to develop abnormally. The types of monsters in these crosses are similar to those produced by treating the eggs with chemical poisons. In many cases these sperm take no part in development but initiate the process by serving as a parthenogenetic agent. Hertwig takes the same position, and further finds, that when the foreign sperm is treated with radium the injurious substance contained in it is killed or destroyed so that the spermatozoon initiates development by parthenogenesis without later causing the development to be abnormal. Bataillon's method of sticking eggs with fine platinum needles to give artificial parthenogenesis is similar, Hertwig thinks, to the use of sperm intensively treated with radium. The treated spermatozoon plays the rôle of the platinum needle in Bataillon's experiment. The male chromatin can no longer combine with the female chromatin, there is no amphimixis. Bataillon, by his sticking method, obtained from 10,000 *R. fusca* eggs only 120 hatched tadpoles, and but three metamorphosed, while in some of Hertwig's radium experiments almost all hatched from the jelly.

The radium experiments of Hertwig give us the first method of artificial parthenogenesis which offers promise for use with mammals. Hertwig suggests that since artificial fertilization is possible in many mammals, one might fertilize with semen which had been intensively treated with radium so that the chromatin was destroyed, and with such sperm artificial parthenogenesis in mammals could be accomplished. Two years before Hertwig made this suggestion Dr. Congdon was trying the effects of radium on the spermatozoa of mice and rats in the anatomical laboratory at Cornell and is now continuing these experiments in the anatomical laboratory at Stanford University; up to now he has not succeeded in obtaining fertilization with the modified spermatozoa, though of course much experimentation is necessary in order to establish the proper intensity of the treatment.

These experiments of Hertwig also afford interesting data as to the nature and importance of the part played by the chromatin in development. The cells of the embryos which resulted from eggs fertilized by intensively treated sperm were found by O. Hertwig, P. Hertwig, and Poll, to contain the reduced number of chromosomes showing that the paternal chromatin had been destroyed by the treatment. Günther Hertwig found the nucleus volume in radium larvæ to be one half the size of the nucleus in the control; he measured the mass of nuclei of nerve cells, liver cells, blood corpuscles, embryonic muscle cells, etc. The entire larva was smaller. P. Hertwig found the male pronucleus derived from intensively treated sperm to be modified in the first and second divisions of the frog's egg and Opperman found the same in the trout. O. Hertwig found in *Triton* eggs that the intensively radiumized male chromatin took no part in the developmental process and the soma cells contained one half the chromosome number. The male chromosome set falls out of the development and the soma nuclei contain only the female set.

Finally, Hertwig obtained another most striking result which may be mentioned, although it is not entirely in line with the present subject. When eggs instead of the spermatozoa were subjected to intensive treatments of 2 to 5 hours with radium, the chromatin of the female pronucleus was found to be broken down and destroyed. If eggs, after such intensive treatment, were fertilized by normal sperm, it was found that they developed almost normally, although when eggs were treated from 15 minutes to $\frac{1}{2}$ hour they always developed abnormally though fertilized with normal spermatozoa. Hertwig, therefore, concludes that the intensively treated eggs fertilized by normal sperm develop by the process of merogony; that is, the egg nucleus being destroyed by the treatment, the sperm nucleus enters the egg and causes development to proceed in the same way that the female pronucleus acts in parthenogenesis. Only one set of chro-

mosomes, either the paternal or maternal, is necessary for development of the egg.

During the summer of 1912 I treated the spermatozoa of fish with various salts and organic substances with negative results. When the treatment was sufficiently strong to affect the spermatozoa it rendered them incapable of fertilizing the eggs. A method could no doubt be devised for modifying fish spermatozoa with various chemicals and of course radium does modify the fish sperm as Opperman found.

Only a few experiments have been performed in attempting to modify the offspring of birds by injuring the male. Todde found that the offspring from alcoholized roosters were not quite normal and that the roosters did not succeed as well as usual in fertilizing eggs. Lustig's experiments showed that by inoculating fowls with abrin the offspring were rendered less resistant to inoculations of abrin than were control animals of the same age. This result followed the inoculations of either parent, the male as well as the female.

A more extensive literature bears upon the actions of poisons on the male germ cells of mammals, though most of the cases are uncontrolled observations. The treatment of the germ cells of mammals is a more complex proposition than the experiments on those lower forms in which the fertilization is external and where, for this reason, the eggs and spermatozoa may be treated directly. In mammals the stimulus must be applied through the animal body and the case is thus complicated since it is often impossible to differentiate between the direct action of the substance applied and the secondary effects due to the responses of the parental body to the treatment. With certain treatments, however, the case is not so complex as would appear at first sight, since the substances may pass into the blood stream and the lymph and act directly on the germ cells just as they do on other tissues and cells of the body.

In experiments to modify the germ cells of mammals

the first proposition becomes then, to determine whether the substances used reach the germ cells directly. One of the best substances for such experiments is alcohol, since its action and distribution in the body has been largely studied and since it acts so decidedly to modify the developmental processes, as many workers have found on invertebrates, and as I have shown by treating fish eggs with this substance.

It is a well known and generally accepted fact that alcohol does cause changes and degeneration in many of the tissues of animals and man. The question arises, how, then, can the reproductive tissues, the ova and spermatozoa escape? Nicloux and Renault have found that alcohol has a decided affinity for the reproductive glands. In the testicular tissues and the seminal fluid an amount of alcohol is soon present which almost equals that in the blood of an individual having recently taken alcohol. The proportion of alcohol in the testis as compared with that in the blood was as 2 to 3, and in the ovary of female mammals as 3 to 5. From these observations it must follow that alcohol may act directly on the ripe spermatozoon shortly before it fertilizes the egg, and if this substance injuriously affects the germ cells, then one should expect to find an indication of the injury in the resulting development as Hertwig has found from his radium treated spermatozoa.

There are a number of observations on human beings bearing on this point, though they probably all need confirmation by experimentation on lower mammals. Lipich claims to have observed 97 children resulting from conception during intoxication. Only 14 of these were without noticeable defects. Twenty-eight were scrofulous, three had "weak lungs," three showed different atrophic conditions, one watery brain, four feeble-minded, etc. Sullivan reported seven fairly authentic cases of drunkenness during conception; six of the offspring died in convulsions after a few months, and the seventh was stillborn.

Rösch was the first, in 1837, to study the reproductive glands of alcoholics and found degeneration of the testicles. Lancereaux described a parenchymatous degeneration of the seminal canals. Simmonds (1898) found azoospermie (spermatozoa without tails) in 60 per cent. of cases of chronic alcoholism; 5 per cent. of these men were sterile. Kyrle reported three cases of total atrophy of the testicular parenchyma in which death had resulted from cirrhosis of the liver due to alcohol. He attributed the atrophy of the testicle to the cirrhosis of the liver and not to chronic alcoholism.

Bertholet (1909) has made an extensive examination of the influence of alcohol on the histological structure of the germ glands, particularly on the testicles of chronic alcoholics. He found testicular atrophy in alcoholics with no cirrhosis of the liver. Bertholet observed partial atrophy of the testicles in the majority of 75 chronic alcoholics. The men died between the ages of 24 and 57 years, the greatest mortality being between 30 and 50 years. In 37 cases, excluding syphilitics, a microscopical examination showed a more or less diffuse atrophy of the testicular parenchyma and a sclerosis of the interstitial connective tissue. The canals were reduced in size and their lumina obliterated. Spermatogonia were atrophic. It was generally impossible to differentiate spermatocytes or spermatids. There were no dividing cells and no spermatozoa. These conditions with slight variations were found in 24 cases. Such atrophic structures were present in one drinker only 29 years old. In 4 cases of cirrhosis of the liver the testicular atrophy had not progressed very far and spermatozoa were still present.

The extreme conditions of atrophy of the testicles were only found in alcoholics. Observing the testicles of non-alcoholics that had died of various chronic illnesses, such as tuberculosis, no atrophy of the testicles or thickening of the membrana propria was found. Two old men of 70 and 91 years still possessed spermatozoa in the canals.

Bertholet concluded that the atrophy he observed was not due to old age, cirrhosis of the liver, or other systemic conditions, but to the effects of chronic alcoholism on the reproductive glands. Weichselbaum has confirmed the observations of Bertholet.

It is certain, however, that the chronic alcoholic is not so often rendered sterile as Bertholet's study would lead one to believe. It is not rare to find alcoholics with large families. My experiments on mammals may not be of sufficient duration at the present time, yet I have male guinea-pigs that have been almost intoxicated on alcohol once per day for six days a week for a period of 32 months, which are still good breeders. Thirty-two months of a guinea-pig's existence is proportionately equal to a good fraction of a human life. A number of these animals have been killed and their testicles examined microscopically and found to be normal. In some cases where the male had failed to succeed in impregnating the female for several times, one of his testicles was removed and studied microscopically; the testicle was found to be normal and the male later gave offspring by other females. Ovaries have been similarly examined and in no case has the alcoholic treatment caused a visible structural change in the reproductive glands. The actual physiological proof of the efficiency of the organs is shown by the ability of the animals to reproduce. Although there is no visible structural change in the germ cells, nevertheless, they have been modified by the treatment to an extent sufficient to cause them in most cases to give rise to defective embryos or weakened individuals which die soon after birth.

Nicloux has carefully demonstrated on dogs and guinea-pigs the passage of alcohol from the blood of the mother into the tissues of the embryo. After a short time the amount of alcohol in the blood of the fetus is about equal to that in the blood of the mother, while there is really slightly more alcohol in a given weight of the tissues of the fetus than is to be found in an equal

weight of liver tissue from the mother. The reality of the passage of alcohol from the mother to the fetus demonstrates the possibility of the intoxication of the fetus.

There is an abundance of data bearing on the effects of parental poisoning on the human offspring, yet almost all of it is complicated. The question arises whether the defects of the offspring are actually due directly to the parental poisoning or to the often degenerate condition of the parent. With lower mammals this question may be controlled, since vigorous individuals with no physical weaknesses may be selected for study. One of the most interesting human cases is that Forel cites as recorded by Schweighofer. A normal woman married a normal man and had three sound children. The husband died and the woman married a drunkard and gave birth to three other children; one of these became a drunkard; one had infantilism, while the third was a social degenerate and drunkard. The first two of these children contracted tuberculosis, which had never before been in the family. The woman married a third time and by this sober husband again produced sound children. This is a logical experiment, the female was first tested with a normal male and gave normal children; when mated with an alcoholic male the progeny were defective. She was later tested again with a normal male and found to be capable of producing sound offspring. A number of such cases are on record but all are open to the question whether the defective offspring are actually due to the effects of the poison on the parent, or to the fact that the parent may have been weak and degenerate from the beginning.

Other substances than alcohol seem to act directly on the germ cells of man and mammals, and these actions are more important since there is no reason to believe, for some of them at any rate, that they accompany a degenerate condition. Constantine Paul long ago pointed out that the children of lead workers were often defective.

He made the interesting observation that when the father alone was employed in such work his children were affected. In 32 conceptions with such fathers 12 resulted in premature labor and stillbirths, 20 living births occurred but only 3 children survived. Eight died the first year, 4 the second, and 5 the third year.

Mairet and Cambemale in 1888 were the first to experiment on the influence of alcohol on the mammalian offspring. They treated a dog for 8 months with absinthe (11 gr. per day per kilo of animal weight) and paired this alcoholized dog with a normal bitch. Twelve young resulted; 2 were born dead, 3 died within 14 days, and the others died between 32 and 67 days of intestinal catarrh, tuberculosis, etc. In a second experiment, both parents were mated while normal, then the female was treated for 23 days (2.75 to 5 gr. of absinthe of 72 per cent. per day per kilo). Of 6 young 3 were stillborn, 2 had normal bodies though of weak intelligence, while one was very sluggish. The evident criticism against this experiment is that an insufficient number of animals was used and there was no control. It is very difficult to rear pups in a laboratory; when apparently perfectly normal, they often die shortly after birth.

Hodge, in 1897, obtained similar results. From one pair of alcoholic dogs he observed 23 pups, 8 were deformed, 9 were born dead, while only 4 lived. In a control set, 41 individuals lived, 4 were deformed, but there were no stillbirths.

Nice has recently published results of treating mice with alcohol. He finds little, if any, effect of the treatment on the offspring. Considering his method of administering the alcohol and the results obtained, the doses used were probably insufficient to produce effects. It may also be possible that mice are more resistant to alcohol than are other mammals. I have discussed these experiments in a previous communication.

EXPERIMENTS

Three years ago a series of experiments were begun on guinea-pigs with the hope of modifying the type of embryo in mammals so as to produce definite monstrosities as one is able to do with lower vertebrates. This primary object has not been fully accomplished, yet the experiments have demonstrated several significant points and have shown that an alcoholized male guinea-pig almost invariably begets a defective offspring even when bred to a vigorous normal female.

Normal, healthy animals are selected for the experiment, and in all cases they are first tested by a normal mating in order to establish their ability to produce vigorous offspring. After such a test the treatments are begun. During the experiments the treated males and females are mated from time to time with normal animals, and in addition, control matings of normal individuals are made. Some of the specimens are treated with alcohol and ether. These substances were used since they readily act upon animal cells and since I had studied their effects on the development of fish embryos and found them to cause rather definite and easily recognizable defects in the central nervous system and organs of special sense.

METHOD AND TECHNIQUE

In the beginning of the experiments alcohol was given along with the food, but the animals ate less and the food did not apparently agree with them. It was then administered in dilute form by a stomach tube; this method disturbed digestion and seemed to upset the animals considerably. It is certain that alcohol given to animals through the stomach deranges their digestion and appetite to such an extent that the experimenter is unable to determine whether the resulting effects are due to the alcohol, as such, or to the general deranged condition of the animal. When given in the drinking water they take little or none of the water and the treat-

ment is insufficient. For these reasons an inhalation method of treatment has been resorted to which, as far as experience goes, has no serious disadvantages and does not complicate the conditions of the experiment.

A fume tank of copper is made of sufficient size to supply breathing space for 4 or 5 guinea-pigs at one time. The tank is arranged with four outlets, so that definite amounts of the fumes may be passed through in a given time and the ventilation controlled. In this way each animal could be given about the same amount of the substances. The individuals, however, differ so in their resistance to the treatment that it has been found better to treat all to about the same degree of intoxication. This physiological index is more reliable as each animal is thus affected in a similar fashion each day. For this purpose they are placed in the fume tank on a wire screen, and absorbent cotton soaked with alcohol is placed beneath the screen, and the animals inhale the fumes. The tank was described and illustrated in a previous article.

Ether is given in a similar manner, except that the animals are much more readily overcome and must be carefully watched while inhaling even the most dilute doses.

In order to avoid handling the females during late pregnancy, a special treating cage is devised. An ordinary box-run with a covered nest in which the animal lives is connected by a drop-door with a metal-lined tank, having a similar screen arrangement to that of the general treatment tank. The pregnant animal may be driven daily into the tank and thus treated with alcohol fumes throughout her pregnancy without being handled in any way that might disturb the developing fetus.

DIRECT EFFECTS OF THE TREATMENT ON THE ANIMALS

Many of the animals have now been treated almost to the point of intoxication for six days per week for nearly three years. They are affected by the alcohol fumes in

different ways; certain ones become drowsy and stupid, while others become excited and sometimes vicious during the treatment, constantly fighting and biting at others in the tank. One male always had to be treated alone on this account. The fumes are inhaled into the lungs and pass directly into the circulation, so that the animals show signs of intoxication very soon after being put into the tank, yet the intake of alcohol is so gradual that they may remain for one hour or more without becoming totally anesthetized. The mucosa of the respiratory tract is considerably irritated during the first few days or weeks of the treatment, but later becomes hardened and little effect can be noticed. The cornea of the eye is greatly irritated and often becomes milky white and opaque during the first few months; but later this clears up in most of the specimens and the animal is able to see perfectly, though one male that has been treated for 32 months is now entirely blind. The general condition of the animals under the treatment is very good; they all continue to grow if treated before reaching their full size, and become fat and vigorous, taking plenty of food and behaving in a normal manner in every particular.

Certain of the animals have been killed at different times during the experiment and their organs and tissues studied microscopically; all have seemed entirely normal. The tissues of one female were examined after she had been treated for over a year, and the heart, stomach, lungs, liver, kidney, etc., were all normal. She was generally fat but there was no fatty accumulation in the parenchyma of any of the organs except possibly a slight excess in the adrenal glands.

As mentioned above several of the animals, both males and females, have been partially castrated during the experiments and the ovaries and testis have been found to be in healthy condition.

The treated animals are, therefore, little changed or injured so far as their behavior and structure goes. Nevertheless, the effects of the treatment are most

decidedly indicated by the type of offspring to which they give rise, whether they are mated together or with normal individuals.

EFFECTS ON THE OFFSPRING

The animals have been mated in various combinations. First, treated males have been paired with normal females, the paternal test; this is the crucial test of the influence of the treatment on the germ cells. In this case the chemically modified or weakened spermatozoon can alone be responsible for the defective offspring, since the egg is normal and develops in a normal environment in the healthy mother.

Second, treated females are paired with normal males, the maternal test. This combination offers two chances for injuring the offspring. Either the ovum may be defective as the result of the treatment which the mother has undergone, and may thus give rise to a defective individual; or secondly, the developing embryo may be affected directly by the alcohol in the system of the mother, since Nicloux has shown that this substance may pass from the blood of the mother into the tissues of the fetus. Thus the intoxication of the embryo may modify its forming structures in the same way that a fish embryo develops deformed organs and parts when in sea-water to which alcohol has been added.

The third combination is the mating of two alcoholic individuals. This is the most severe test and offers the greatest chance for defective offspring.

Before the experiment or treatment begins the guinea-pigs are all tested by normal matings and are found to give normal vigorous offspring. They continue to give normal offspring until the treatment has lasted for some time. The effect accumulates slowly and is not noticed at once. A number of experiments in which the treatment of a female was commenced at the beginning of pregnancy have so far given rather indefinite results, although a slight effect may be indicated.

In all 124 matings of treated individuals have been made. One hundred and three of these have reached full-term and are recorded. Twenty-one matings are not yet due. From the 103 full-term matings only 52 young have survived and most of these are somewhat under size and show their affected condition in the type of offspring to which they give rise. Yet their parents were all unusually large and originally strong animals.

From 35 control matings 56 healthy offspring have been derived which continue to produce normal animals in the following generations, in a few cases now to the fourth generation.

A tabulated summary of the results may be arranged as indicated in Table 1. The conditions of the animals in the mating pairs are shown in the first column of the table and the total results of the matings are indicated in the following columns.

The first horizontal line gives the record when alcoholic males are paired with normal females. Fifty-nine such matings have reached term, 25 of these gave negative results or early abortions. Some embryos were aborted during very early stages and were generally in such poor condition when found in the cages that little could be learned from them. They were partially or completely eaten by the mother in most cases. The males were always kept with the females during favorable periods for a number of days, usually about three weeks, and conception should have occurred in all cases, as it did with the control matings.

Thirty-four of the 59 matings resulted in conceptions which ran the full term. Eight, or about 24 per cent. of these, were stillborn litters, consisting in all of 15 individuals. Most of these were somewhat premature; in a few cases their eyelids were still closed and the hair was sparse on their bodies. (A normal guinea-pig at birth is well covered with a hairy coat, its eyelids are open and it very quickly begins to run about.)

Twenty-six, or only 44 per cent., of the matings pro-

duced litters of living young. These litters contained in all 54 individuals. Twenty-one, or almost 40 per cent., of these young guinea-pigs died within a few days or less than four weeks after birth, while 33 of them survived. *Thus, out of 69 full term young, of which 54 were born alive, only 33 have survived, and many of these are small and excitable animals, and although not treated themselves have since given rise to defective offspring in several cases where they have been mated with one another.* On the other hand, 35 control matings have produced 32 living litters consisting of 60 individuals, only 4 of which have died and 56 are perfectly normal animals.

It is of interest that the young animals before dying show various nervous disturbances, having epileptic-like seizures, and in most cases die in a state of convulsion.

The important fact in the above case is that the father only was alcoholic, the mother being a normally vigorous animal. *This experiment clearly demonstrates that the paternal germ cells may be modified by chemical treatment to such a degree that the treated male will beget abnormal offspring even though he be mated with a vigorous female.* A reconsideration of the figures in the first line of the table shows really how decidedly the injured spermatozoon expresses itself in the fate of the egg with which it combines.

For comparison the second line of the table shows the results of matings between alcoholized females and normal males. These matings might be expected to give more marked results than the previous ones, since in the treated female not only the germ cells may be affected, but the developing embryo itself may be injured by the presence of alcohol in the blood of the mother.

There are 15 matings between alcoholized females and normal males. Three of these, or 20 per cent., gave negative results, or were possibly aborted very early. Three stillborn litters were produced consisting of nine individuals, while 60 per cent. of the matings gave living litters. This result is better by 16 per cent. than that

obtained when alcoholized males were paired with normal females. The proportion of surviving individuals is, however, less from the treated females than from the treated males. The 9 living litters contained 19 young, 9 of which died soon after birth and 10 survived. Thus out of 28 full term young only 10, or about 36 per cent., survived, while 64 per cent. of the offspring were lost; in the above cases where the male alone was alcoholic almost 48 per cent. of the full term young survived.

TABLE I

CONDITION OF THE OFFSPRING FROM GUINEA-PIGS TREATED WITH ALCOHOL

Condition of the Animals	Number of Matings	Negative Result or Early Abortion	Still-born Litters	Number Still-born Young	Living Litters	Young Dying Soon After Birth	Surviving Young
Alcoholic ♂ by normal ♀	59	25	8	15	26	21	33
Normal ♂ by alcoholic ♀	15	3	3	9	9	9	10
Alcoholic ♂ by alcoholic ♀ . . .	29	15	3	6	11	7	9
SUMMARY	103	43	14	30	46	37	52
Normal ♂ by normal ♀	35	2	1	4	32	4	56
2d generation by normal	3	0	0	0	3	0	4
2d generation by alcoholic	3	0	2	5	1	0	2
2d generation by 2d generation	19	7	0	0	12	6	13
Female treated during pregnancy	4	0	0	0	4	1	7

The third horizontal line indicates the results of pairing alcoholized males with alcoholized females. The effects of the treatment in this case are slightly more marked than in either of the above lines. Twenty-nine such matings gave in 15, or more than 50 per cent., of the cases negative results or early abortions. Three stillborn litters occurred, each consisting of two individuals. Only 11 living litters were produced. These contained 16 young, 9 of which survived while 7 died soon after birth.

A comparison of this combination with the control matings given in the fifth line shows in a decisive manner the really detrimental effects of the treatment. In the one case only 9 surviving young were obtained from 29

matings, while in the other the control animals gave 56 surviving young from 35 matings.

The fourth line summarizes the results of the matings made with treated individuals. A total of 103 matings have run the full term; 43, or almost 42 per cent. of these, have given negative results or early abortions; while 35 control matings failed in only two cases to yield a full term litter. Fourteen, or $13\frac{1}{2}$ per cent., of the matings gave stillborn litters consisting of 30 individuals. Only one stillborn litter occurred in the 35 control matings; this was a large litter of 4 young and the mother seemed almost unable to carry them. The 103 matings of treated animals gave only 46 living litters, about 45 per cent., while 32 living litters, or $91\frac{1}{2}$ per cent., were produced by the 35 control matings. The 46 living litters from the alcoholic individuals contained 89 young, 37 of which died shortly after birth and 52 survived. The 32 living litters from the normal animals consisted of 60 individuals, only 4 of which died while 56, or 93 per cent., of these survived.

Of 119 full term young, 30 of which were stillborn, produced by the alcoholic animals, only 52, or less than 44 per cent., survived as against the 56, or $87\frac{1}{2}$ per cent., survivors among the 64 full-term control offspring.

The bottom line of the table shows that 4 normally mated females treated with alcohol during the period of gestation gave 4 living litters, consisting each of 2 young. One out of the 8 young died soon after birth. These few cases would seem to indicate that the treatment, when started at the beginning of gestation, was not particularly injurious to the embryos developing *in utero*.

ARE THE EFFECTS ON THE OFFSPRING TRANSMITTED?

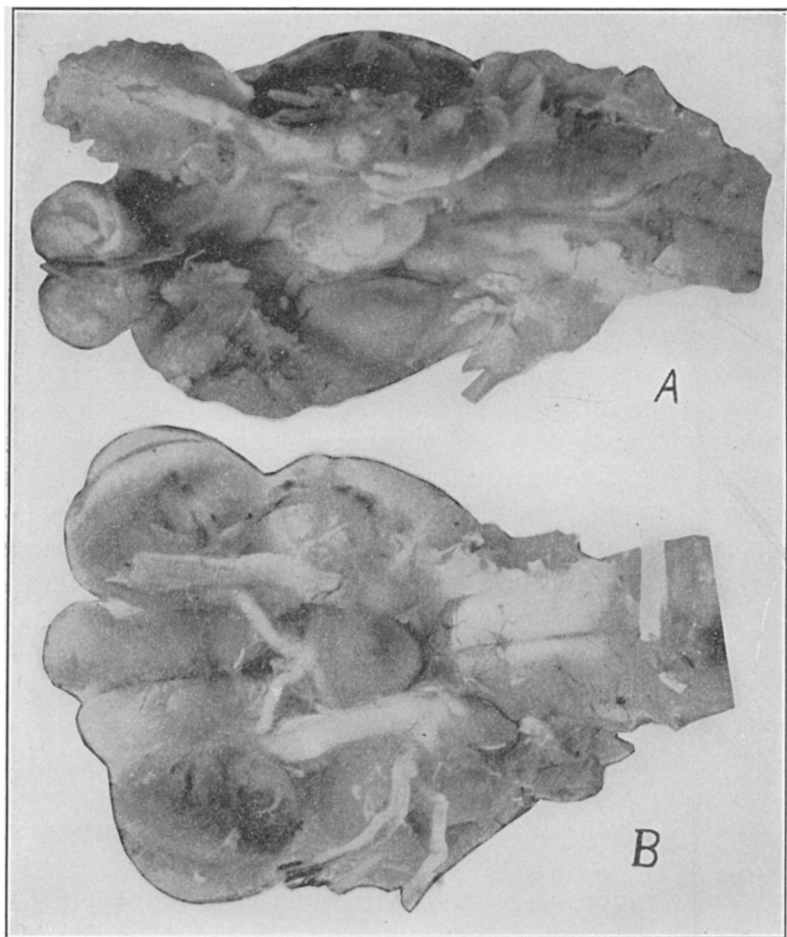
The offspring derived from the alcoholic individuals are termed second generation animals, and were not treated with alcohol. The sixth, seventh and eighth lines of the table represent the data obtained from the few full time matings that have been made with these guinea-pigs.

In three cases second generation animals have been mated with normal individuals and have produced perfect results, though the litters have been small. Three litters are recorded containing a total of 4 young, all of which survived. It might seem as though the normal mate counteracted any defect which may have been present in the second generation animals.

The mating of second generation individuals with alcoholized guinea-pigs gave decidedly different results. The seventh line shows that 2 out of 3 such matings produced stillborn young. In one of these cases the female was of the second generation and the male alcoholic, and in the other case the reverse condition existed; yet both combinations gave dead offspring, one litter of two and one of three individuals. One of these specimens from the second generation female by the alcoholic male was grossly deformed. The third mating gave two surviving young. At present there are too few matings of this combination from which to draw conclusions, yet the results obtained are the most disastrous of all.

Nineteen matings between second generation animals have been made. The outcome in these cases compares very unfavorably with that from the control matings, while the data are much of the same type as those obtained from the alcoholic combinations. Seven, or almost 37 per cent., of the matings gave negative results. Twelve living litters were born consisting of 19 individuals, 6, or about 32 per cent., of which died very soon after birth and showed various nervous disorders. One was entirely eyeless and decidedly deformed.

From the number of records available one might conclude that the effects of the alcoholic treatment were as pronounced upon the offspring of the second generation animals, although they had not been directly treated, as they were upon the offspring of alcoholized individuals. The poison acts upon the cells and tissues of the body, the germ cells as well as other cells, and an offspring derived from the weakened or affected germ cell has all



EXPLANATION OF PLATE

Ventral surfaces of two guinea-pig brains. *A*, the brain of an animal which was entirely eyeless, no optic nerves or tracts are present. *B*, the brain from another member of the same litter. This animal was defective and died shortly after birth, but possessed normal eyes as the photograph shows and the optic nerves are clearly seen passing medially to form the chiasma and from there the optic tracts are shown along the anterior margins of the tuber cineria. All of these optic structures are missing in the brain above.

the cells of its body, both soma and germ, defective since each of the cells is a descendant of the injured germ cell combination.

These are the initial experiments with mammals to show *that an injury of the germ cells may express its effects on the offspring and be passed through subsequent generations.*

The actual outcome of the experiments may be more fully recognized by a consideration of one of the most striking cases. A large normal female, weighing about 700 grams, had given two normal young by a control mating and had since given non-viable young by a mating with an alcoholic male. She was then mated with another large strong alcoholic male which weighed 740 grams and which had given before this mating apparently healthy offspring by normal females. The mating resulted in the production of 4 young, all small and rather excitable in their behavior. These individuals from the normal mother and the alcoholized father grew slowly, although they ate freely and appeared to be well. They remained small and below the average in weight. Three were males and one was a female.

One of the males was mated with a normal female and two normal young resulted. He was then mated with a female from an alcoholic father and she gave birth to two small young; one of these offspring was only half size and very excitable. He was then mated with a female from an alcoholic mother and one small young was produced.

A second one of the three males was mated with a normal female which produced one large apparently normal offspring. He was then mated with a female from an alcoholic father and two small young resulted, one of which died within five days and the other is weak and nervous. He was again mated with a normal female and one normal young was produced.

The third male was mated with his sister and she gave birth to 3 young. One of the young died when one day

old, having been in a constant tremor since its birth; another lived for nine days but whenever it attempted to walk it was seized with spasmodic contractions; the third specimen exhibited the same nervous manifestations and was completely eyeless. This animal died eight days after birth and an examination of the brain showed an entire absence of optic tracts, as may be seen in Plate 1A.

In the development of this animal it is probable that the optic vesicles were suppressed and never arose from the brain. Thus, no eyes, optic nerves, or optic tracts could have formed. This particular eyeless condition in these experiments is of interest since one is readily able to suppress the origin of the optic vesicles in fish and chick embryos by similarly weakening the embryo with treatments of alcohol, ether, etc.

The mother of these offspring was remated with her brother, but she died six weeks later, not becoming pregnant. She was in an emaciated condition but had always been less than half normal weight.

The three extremely weak and defective offspring were doubtless due to the fact that both of their parents had similarly weakened or injured constitutions, having resulted from a single mating of a normal female with an alcoholized male. The eyeless offspring and the other two nervous non-viable individuals should not be interpreted as due merely to the fact that their parents were brother and sister. Several normal matings of brother and sister have been made during the experiment and perfectly healthy offspring have been produced. In the studies of heredity conducted on guinea-pigs brother and sister are crossed with impunity, in no way weakening their offspring. The significant point in the present consideration is that the two animals coming from the same mother and treated father may have had similar weaknesses or defects and the combination of two such individuals resulted in offspring which exhibited these defects to a more decided extent. The three animals were far more defective than their parents and owed their defects

to the modified condition of the germ cells of the grandfather from which they descended.

CONSIDERATION OF INDIVIDUAL MATINGS AND RESPONSES

Studying the data of individual animals, another point of some importance presents itself. This is the fact that the same male often yields very different results when bred to different females, although the females are similar so far as the experiment goes, being either all normal or alcoholic. This may be due to the varying degrees of resistance or hardiness possessed by the germ cells of different individuals. A part of this difference may be due to the fact that as the treatment advances the germ cells are more affected and the results do actually become more pronounced.

Male No. 5 that has been treated with alcohol for two and one half years, has been mated 25 times. Eleven of these matings have yielded negative results; 4 were with alcoholic females and 7 were with normal. Three of the matings gave stillbirths, 2 with normal females and one by an alcoholic female. Only 11 of the 25 matings gave living litters, 4 of these were non-viable and 7 litters survived. Five of the 7 surviving litters were from normal females and 2 from alcoholic mothers. *This alcoholic male has, therefore, begotten only 7 surviving litters out of the 14 full term litters born and out of a total of 25 matings.*

Alcoholic male No. 6 shows a still worse record. Out of a total of 21 matings, 10 have given negative results, 5 by normal and 5 by alcoholic females. Two matings gave stillborn litters, one from a normal female and one from a second generation female. Nine living litters resulted from the 21 matings, 3 from alcoholic females and 6 from normal. Five of the nine litters born alive were non-viable, the young dying soon after birth. *Only 4 litters survived out of the 11 reaching term and out of the total of 21 matings.* Three of these surviving litters were from normal females and one was from an alcoholic mother.

Alcoholic male No. 43 has been mated 10 times. Three of the matings gave negative results, two by alcoholic females and one by a normal. One stillborn litter resulted from a mating with an alcoholic female. Six living litters came from the 10 matings. The young in 3 of these litters died soon after birth and 3 litters survived.

Alcoholic male No. 45 has now been treated about one and one half years and has been mated 9 times. One mating with a normal female gave a negative result. One mating with a normal female gave a stillborn litter. Seven living litters were produced, 5 of which survived, 2 from alcoholic females and 3 from normal females. The data in this case appear slightly better than in the foregoing but this is due to the fact that only a few matings have been made and most of these during the early stages of the treatment, when the effects are not so pronounced.

All of the other alcoholized males in the experiments show comparable records.

A reference to Table I shows that with three exceptions 35 matings of normal males with normal females gave living litters containing in all 60 individuals, only 4 of which failed to survive. This record stands in striking contrast to the data recorded above from the 4 alcoholic males, and it shows convincingly that the alcoholic treatment has affected the germ cells of these males so that they are no longer capable of producing entirely normal offspring even though they be mated with normal females.

The outcome of the successive matings of fifteen different females is tabulated in Table II. The varying ways in which the same individual has responded in different matings is noticeable. Number 15, a normal female, shows an instructive record. Mated with alcoholic male No. 6 she gave two stillborn young; mated with alcoholic male No. 5 a negative result; remated with No. 5, two young were born and both died of convulsions during the fourth week; then mated with a normal male a normal vigorous offspring was produced; mated again with alco-

holic male No. 5 an apparently normal guinea-pig was born; mated with No. 43, an alcoholic male, she gave 2 young which only lived for two days; then mated again with a normal male she produced 2 vigorous offspring and finally mated with No. 69, an etherized male, 2 young were born, one of which died at birth. Thus, out of the 8 matings, 2 with normal males gave perfectly normal offspring, while 5 out of the 6 matings with treated males gave disastrous results and only one of these matings resulted in the production of an apparently normal young.

Number 56, a normal female, mated to a normal male, No. 48, gave 2 normal young; with alcoholic male No. 45 gave 3 premature stillborn fetuses; again to a normal male No. 80 gave 3 normal offspring; and finally again to alcoholic male No. 45 she gave one very small young.

Normal female No. 63 gave two normal individuals by a normal mating and then three successive matings with an alcoholic male failed to produce a viable offspring; one mating resulted negatively, one gave three young dying shortly after birth, and in the third case two late fetuses were aborted. She was then mated again to a normal male and produced two vigorous offspring.

Normal female No. 50 was mated alternately with normal and alcoholic males for 4 matings, with alternately good and bad results.

Animals 19, 30, 54 and 58, all normal females, show records closely similar to the ones just mentioned. (In a former table of successive matings the first mating of No. 19 has been recorded incorrectly; it should read by a normal male giving two normal offspring as in the present Table II, instead of by alcoholic male No. 4 with a negative result.)

Alcoholic female No. 64 gave two normal young by a normal male before her treatment was commenced. Mated to alcoholic male No. 6 she gave two young; one died at birth and one survived; with alcoholic male No. 5 she gave an apparently normal young; finally, by alco-

TABLE II
RESULTS OF THE SUCCESSIVE MATINGS OF FIFTEEN FEMALES

Animal	1st Mating	2d Mating	3d Mating	4th Mating	5th Mating	6th Mating	7th Mating	8th Mating
No. 64 alc.	Nor. ♂, 2 nor. young	Alc. ♂ 6, 2 young, 1 died	Alc. ♂ 5, 1 nor. young	Alc. ♂ 43, 2 foetuses, died <i>in utero</i> , killed mother Alc. ♂ 43, 0
No. 59 alc.	Nor. ♂, 3 nor. young	Nor. ♂ 44, 2 young, 1 died	Alc. ♂ 43, 0	Nor. ♂ 69, 3 yg., died <i>in utero</i> , killed mother
No. 30 nor.	Nor. ♂ 25, 2 nor. young	Alc. ♂ 45, 2 nor. young	Alc. ♂ 5, 1 nor. young	Alc. ♂ 5, 2 foetuses, died <i>in utero</i> , killed mother Nor. ♂, 1 nor. young
No. 15 nor.	Alc. ♂ 6, 2 still-born	Alc. ♂ 5, 0	Alc. ♂ 5, 2 young, both died in convulsions Alc. ♂ 6, 0	Alc. ♂ 5, 4 small, ½ size young Alc. ♂ 5, 2 late foetuses aborted Alc. ♀ 43, 0	Alc. ♂ 5, 1 apparently normal Alc. ♂ 45, 2 young, 1 died Nor. ♂ 93, 2 nor. young	Alc. ♂ 43, 2 young, both died 2d day Alc. ♂ 6, 0	Nor. ♂ 70, 2 nor. young Alc. ♂ 6, 3 young, 2 died	Ether ♂ 69, 2 young, 1 died
No. 19 nor.	Nor. ♂, 2 nor. young	Alc. ♂ 6, 1 still-born	Alc. ♂ 5, 3 young, all died	Alc. ♂ 5, 2 late foetuses aborted Alc. ♀ 43, 0
No. 63 nor.	Nor. ♂ 44, 2 nor. young	Alc. ♂ 5, 0	Alc. ♂ 5, 3 young, all died	Alc. ♂ 5, 2 late foetuses aborted Alc. ♀ 43, 0
No. 50 nor.	Nor. ♂ 46, 3 nor. young	Alc. ♂ 43, 2 young, both died	Nor. ♂ 69, 2 nor. young	Alc. ♂ 43, 0
No. 54 nor.	Nor. ♂ 44, 2 nor. young	Alc. ♂ 6, 2 young, both died	Nor. ♂ 72, 2 nor. young	Alc. ♂ 43, 1 young

Animal	1st Mating	2d Mating	3d Mating	4th Mating	5th Mating	6th Mating	7th Mating	8th Mating
No. 55 alc.	Nor. ♂, 2 nor. young	Alc. ♂ 6, 0	Nor. ♂ 47, 2 young, both died	Alc. ♂ 6, 1 young
No. 56 nor.	Nor. ♂ 48, 2 nor. young	Alc. ♂ 45, 3 premature, stillborn	Nor. ♂ 80, 3 nor. young	Alc. ♂ 45, 1 young, very small
No. 58 nor.	Nor. ♂ 46, 2 nor. young	Alc. ♂ 6, 2 nor. young	Alc. ♂ 43, 1 young, died at birth	Alc. ♂ 6, 0
No. 60 alc.	Nor. ♂ 46, 3 young, all died	Nor. ♂ 44, 2 nor. young	Alc. ♂ 45, 1 stillborn	Alc. ♂ 43
No. 62 alc.	Nor. ♂ 47, 2 nor. young	Nor. ♂ 46, 2 small young	Alc. ♂ 6, 2 young, died 2d day	Alc. ♂ 45, 2 young, died 2d day
No. 65 nor.	Nor. ♂, 2 nor. young	Alc. ♂ 5, 2 small young	Alc. ♂ 6, 2 small young	Nor. ♂ 83, 2 young, 1 died, ♀ treated during pregnancy
No. 66 alc.	Nor. ♂, 1 nor. young	Nor. ♂ 44, 2 nor. young	Alc. ♂ 5, 0	2d gen. ♂ 102, 3 fetuses aborted	2d gen. ♂ 102, 2 small young

holic male No. 43 she produced two fetuses which died *in utero* and killed her.

Female No. 59, alcoholic, has a similar record and was also finally killed by 3 fetuses dying *in utero* and poisoning her.

Alcoholic females 55, 60, 62 and 66 all show very poor records in the production of viable offspring.

During the course of the experiments four females have been killed by the death of fetuses *in utero*. In three of the cases this occurred after the females had been treated with alcohol for a number of months and were becoming more and more affected by the treatment. No. 61 died after she had been given alcohol for four months with three fetuses *in utero* which had apparently been dead for several days. No. 64 had been treated with alcohol for over one year and finally, while in a late stage of pregnancy by alcoholic male No. 43, she became stupid and refused to eat. On examination the fetuses showed no signs of life and were quite hard; they were removed by operation and had been dead several days. The mother had become so intoxicated by this condition that she was unable to recover after the removal of the fetuses.

Female No. 59 had been treated with alcohol for thirteen months when, after being mated with a normal male, she was operated upon in order to remove three dead fetuses. She failed to recover.

The fourth case of young dying *in utero* was that of a normal female, No. 30, that had been mated with an alcoholic male. The almost full term fetuses died and produced the same symptoms in the mother as those in the cases above; she was also operated upon and failed to recover.

It is a perfectly easy operation to remove the ovaries or uterus from a normal guinea-pig. I have not tried to remove living fetuses. There is little doubt, however, that it is the accumulated toxins owing to the presence of

the dead fetuses which prevented the females from recovering after the operation in these three cases.

The death of the late fetuses *in utero* is to be expected merely as a step in the series. A number of early abortions of embryos have occurred, and the table shows the enormous fatality among the young shortly after birth, as well as the frequent occurrence of stillborn litters. When the young happen to die shortly before birth instead of after birth, the female in some cases is unable to expel them from the uterus.

A consideration of a few of the notes made from the individual matings will further serve to illustrate the actual response of the animals to the treatment as shown by the outcome of the matings. For this purpose we may take two random groups.

First a group of eleven matings made on October 30 and 31, 1912, resulted as shown in the following notes:

Oct. 30, Nor. ♀ No. 30 \times Alc. ♂ 5 = Jan. 9, 1913—One normal young, No. 123 ♀.

Oct. 30, Nor. ♀ No. 29 \times Alc. ♂ 45 = Jan. 20, 1913—Three very small young, Nos. 134 ♂, 135 ♀, and 136 ♀.

Oct. 30, Nor. ♀ No. 58 \times Alc. ♂ 6 = Jan. 15, 1913—Two normal young, Nos. 129 ♂, 130 ♀.

Oct. 30, Alc. ♀ No. 62 \times Nor. ♂ 46 = Jan. 9, 1913—Two small but normal young, Nos. 121 ♀, 122 ♀.

Oct. 30, Alc. ♀ No. 59 \times Alc. ♂ 43 = 0.—*Only one of the eleven matings that did not take.*

Oct. 30, Alc. ♀ No. 60 \times Nor. ♂ 44 = Jan. 17, 1913—Two apparently normal young, Nos. 131 ♂, 132 ♂.

Oct. 31, Nor. ♀ No. 68 \times Nor. ♂ 69 = Jan. 21, 1913—Three normal young, Nos. 137 ♂, 138 ♂, 139 ♂.

Oct. 31, Nor. ♀ No. 71 \times Nor. ♂ 72 = Jan. 10, 1913—Two small normal young, Nos. 126 ♂, 127 ♂.

Oct. 31, Nor. ♀ No. 73 \times Nor. ♂ 70 = Jan. 19, 1913—One large young, No. 133 ♀.

Oct. 31, Nor. ♀ No. 74 \times Nor. ♂ 79 = Jan. 11, 1913—One large young, No. 128 ♀.

Oct. 31, Alc. ♀ No. 55 \times Nor. ♂ 47 = Jan. 9, 1913—Two

small and weak young, Nos. 124 ♀, 125 ♂, both died when one month old.

In the group of 11 matings, 4 were between normal males and normal females, control matings, three were between alcoholic males and normal females, and three between normal males and alcoholic females. All of these produced offspring as noted, while the single mating between an alcoholic male and an alcoholic female gave a negative result although the animals were kept together as long as the individuals of any of the other pairs, 17 days.

One of the alcoholic females gave two young which died within a month.

A second group of 18 matings made during November, 1912, are recorded as follows:

Nov. 4, 2d Gen. ♀ 100 \times 2d Gen. ♂ 92 = 0.—Together very long, though failed to take.

Nov. 4, 2d Gen. ♀ 101 \times 2d Gen. ♂ 99 = 0.—Together very long, though failed to take.

Nov. 15, 2d Gen. ♀ 91 \times 2d Gen. ♂ 92 = Feb. 18, 1913—One large young, apparently normal, No. 170 ♂.

Nov. 15, 2d Gen. ♀ 98 \times 2d Gen. ♂ 99 = 0.—Failed to take, though together six weeks.

Nov. 16, 2d Gen. ♀ 76 \times 2d Gen. ♂ 77 = Jan. 28, 1913—Three defective young, one eyeless, all died within 10 days.

Nov. 16, Nor. ♀ 88 \times 2d Gen. ♂ 78 = Feb. 20, 1913—One large young apparently normal, No. 171 ♂ (long gestation).

Nov. 16, Alc. ♀ 66 \times Alc. ♂ 5 = 0.

Nov. 16, Nor. ♀ 87 \times 2d Gen. ♂ 75 = Jan. 27, 1913—Two normal young, Nos. 140 ♀, 141 ♂.

Nov. 16, Nor. ♀ 19 \times Alc. ♂ 6 = Jan. 30, 1913—Three small weak young, two died shortly after birth, one No. 146 ♂ survived.

Nov. 16, Nor. ♀ 34 \times Alc. ♂ 43 = Feb. 4, 1913—Two normal young, Nos. 156 ♀, 157 ♂.

Nov. 16, Nor. ♀ 49 × Alc. ♂ 45 = Jan. 31, 1913—Three normal young, Nos. 147 ♂, 148 ♀, 149 ♀.

Nov. 16, Nor. ♀ 33 × Nor. ♂ 46 = Jan. 30, 1913—Two fine young, Nos. 144 ♂, 145 ♀.

Nov. 23, Nor. ♀ 50 × Nor. ♂ 69 = Feb. 5, 1913—Two large young, Nos. 160 ♂, 161 ♀.

Nov. 23, Nor. ♀ 15 × Nor. ♂ 70 = Feb. 4, 1913—Two large young, Nos. 158 ♀, 159 ♂.

Nov. 23, Nor. ♀ 54 × Nor. ♂ 72 = Feb. 3, 1913—Two large young, Nos. 150 ♂, 151 ♂.

Nov. 23, Nor. ♀ 56 × Nor. ♂ 80 = Feb. 3, 1913—Three normal young, Nos. 152 ♂, 153 ♂, 154 ♀.

Nov. 23, Nor. ♀ 52 × Nor. ♂ 81 = Feb. 12, 1913—One large young, 167 ♀.

Nov. 23, Nor. ♀ 53 × Nor. ♂ 48 = Feb. 3, 1913—One large young, 155 ♂.

To summarize these 18 pairings: 7 were normal control matings all giving vigorous young. Three were alcoholic males by normal females, all gave young. Two litters consisted of small animals, while the third litter was very weak, two of its members died just after birth.

Five of the matings were made between untreated animals which came from alcoholic parents, second generation animals. One litter of 3 individuals was born, all were weak and defective, one being eyeless and the 3 died within ten days. One other litter consisted of only one individual which was born after an unusually long period of gestation. Three of the matings gave negative results. Thus 3 out of 5 second generation matings failed to take, one gave non-viable young, and only one litter of viable young was produced from the 5 matings, as against 7 viable litters from the 7 control matings.

There were two matings of second generation males by normal females. Both of these gave viable young, though one of the females had an unusually long gestation period. The normal mates seemed to have counteracted the weakened condition of the second generation males.

The one mating between two alcoholic individuals again gave negative results.

Thus 4 of the 18 matings failed to take, 3 of these were between second generation animals and the fourth was the double alcoholic mating.

Fourteen matings were successful, in five cases one member of the pair was normal and in seven cases both were normal. In the remaining two cases both animals were of the "second generation," though themselves untreated, one litter was non-viable and but a single litter of one young survived.

These sample notes from 29 pairs out of the total of 167 full term matings contained in Table I, gives a fairly clear idea of the manner in which the individual animals respond.

CONCLUSIONS

Finally, in conclusion, we may consider the type or nature of the injury produced by the treatments and the manner of transmission or inheritance involved. The treated animals themselves show no effects of nervous or systemic injuries in their general health or behavior. It is only when such individuals are bred that they prove to be inferior to the untreated animals. This inferiority is shown both by a slowness or failure in many cases to conceive, although they copulate normally, and by the poor quality of the offspring to which the successful conceptions give rise. That this poor quality of offspring is due to an injury inflicted by the treatment on the germ cells of the alcoholic animals is shown by the fact that when the male alone is treated the offspring he begets are decidedly inferior. The germinal taint is still further demonstrated by the fact that the offspring from treated parents although themselves not treated produce equally or more defective young than do the treated animals.

The defects shown by the offspring of alcoholic parentage are general in type, not definite or specific. The central nervous system and special sense organs are

apparently most affected, and this is true also in embryos developing in unfavorable environments. I have found that fish embryos when developed in a large number of unusual environments, including alcohol and ether, always show marked abnormalities of the nervous system and special sense organs, particularly of the eyes and ears. When chick embryos are subjected to similar environmental conditions, it has been found in experiments performed during the last two winters, that they respond in a manner similar to the fish. Many chick embryos show different degrees of cyclopia and the degeneration or absence of one eye of the normal pair is a common defect in the chick as it is in the fish where many grades of monophthalmicum asymmetricum were described in my communications on the subject. In this connection the eyeless guinea-pig derived from untreated animals that had an alcoholic father becomes of special interest, and the general nervous symptoms, spasms, epileptic-like seizures, etc., shown by animals of two generations gain importance.

All defects of the nature of those mentioned may be considered as due to weakened development or developmental arrest. Any environment which weakens or retards the early stages of development will cause such conditions. How, then, are they transmitted by the alcoholic male, or by the untreated offspring of alcoholic parentage?

When the animal is treated with alcohol, lead or almost any poison for a long period of time, the poison acts to weaken or injure all of the body tissues with which it comes in contact through the circulation, the liver and other glandular organs usually show the effects in particular. The reproductive glands are injured as well as others and all the cells and tissues of such an organ are below normal. When such a male animal is paired with a normal female, the resulting offspring contains in every cell of its body elements derived from the weak or injured male pronucleus. Unless the vigor of the normal parent

is sufficient to overcome the injured condition, the offspring is defective.

The important thing in considering this defective offspring is the recognition of the fact that not only its soma cells but its germ cells as well are defective, since all were derived from the modified spermatozoon of the injured father. When this offspring with injured germ cells is paired with a similar individual, as has been frequently done in the experiments described, the resulting animal body is constituted of cells, all of which are the result of proliferation or division from the primary injured egg and sperm cell; thus all of the cells are of a similar inferior nature. Therefore, the young derived from the second generation should be, leaving out of consideration the power of a cell to recover from such poisoning, equally as defective as those derived from the treated parents.

This might be construed to show the transmission of acquired characters, but it can not be properly interpreted in such a sense. There is in this case no transmission of new or strange characters strictly speaking, merely a weakened or injured cell gives rise to other weak cells. The term "weak" is employed for the lack of a better one, meaning that the cells are below normal in reaction, respond slowly or in a deranged manner and often die or wear out early in their career.

It may be that in nature such defects as hare-lip and cleft palate are transmitted in a fashion similar to the method just suggested. These defects run in families and are said to be inherited. Their character, however, is clearly that of a developmental arrest. Such defects are very probably not truly inherited at all, that is, they are not definite characters or qualities as hair and eye color are, but are due to the fact that the germ cells from which the deformed individual arose, or the uterine environment in which it developed, were not fully normal in vigor. A more careful study of the inheritance of such defects will doubtless reveal the fact that other deform-

ities and developmental arrests are also common in the same families. In other words, weak germ cells or the poor developmental environment runs in the family, and hare-lip and cleft palate are merely the external expressions of these conditions.

The interpretation may be concretely expressed as follows: Mammals treated with injurious substances, such as alcohol, ether, lead, etc., suffer from the treatments by having the tissues of their bodies injured. When the reproductive glands and germ cells become injured in this way they give rise to offspring showing weak and degenerate conditions of a general nature, and every cell of these offspring having been derived from the injured egg or sperm cell are necessarily similarly injured and can only give rise to other injured cells and thus the next generation of offspring are equally weak and injured, and so on. The only hope for such a line of individuals is that it can be crossed by normal stock, in which case the vigor of the normal germ cell in the combination may counteract, or at any rate reduce, the extent of injury in the body cells of the resulting animal. By continually introducing normal mates into such a line the defects might be entirely eliminated, but the continued interbreeding of animals with defects or systemic injuries will doubtless result in the death of the race.

The offspring from a diseased father derives all of its cells from the poor sperm, thus each cell is poor in part and is so passed from generation to generation.

The present experiments are being continued and a large number of matings between second and third generation animals are now made. Various combinations of second generation animals are being tried in order to compare the effects resulting from paternal and maternal treatments, as well as the doubled effects. Two animals, both derived from alcoholic fathers, are mated, others from alcoholic mothers, and the various crosses between these classes are tried. In other cases second generation sisters are mated one with a normal and the other with

an alcoholic male, and subsequently these matings will be reversed in order to study the power of the normal mate to counteract the injured condition, as well as the tendency of new alcoholic cells to augment the condition.

SUMMARY

Three years ago a series of experiments were begun with guinea-pigs in order to test the possibility of modifying the type of development in mammals, so as to produce definite monstrosities, as had been accomplished with lower vertebrates. This primary object has not been fully attained at the present time, yet the experiments have demonstrated several points concerning injury of the germ cells, and have shown that an alcoholized male guinea-pig almost invariably begets defective offspring even when mated with a vigorous normal female.

A method has been devised for administering the alcohol by inhalation. The animals inhale the fumes of 95 per cent. alcohol which are readily taken into the pulmonary circulation, and very soon cause a state of intoxication. By this method the stomach is not injured and the general metabolism of the animal is maintained in a healthy condition. Few changes are produced in the tissues of the animals, even after a treatment given six times per week has extended over almost three years. Yet the actual effects upon the reproductive glands are indicated by the inferior quality of the offspring to which the alcoholized individuals give rise.

The animals have been mated in various combinations. First, alcoholized males are paired with normal females, the paternal test, and also the crucial test of the influence of the treatment on the germ cells. Fifty-nine such matings have reached term. Twenty-five of these gave negative results or early abortions. Thirty-four of the fifty-nine matings resulted in conception which ran the full term. Eight, or about 24 per cent., of these were stillborn litters containing in all 15 dead individuals. Many of them were somewhat premature. Twenty six, or only 44

per cent., of the matings produced litters of living young, containing a total of 54. Twenty-one, or almost 40 per cent., of these young animals died within a few days or less than four weeks after birth and only 33 of them survived. Many of the 33 survivors are small excitable animals and though not treated themselves have usually given rise to defective offspring in the several cases where they have been mated with one another.

The second combination is between alcoholized females and normal males, the results of which are interesting in comparison with the above. In this combination there are two chances to injure the offspring; in the first place it may arise from a defective egg cell, or secondly, it may be injured by an abnormal developmental environment within the body of the alcoholized female. Fifteen such matings have been made. Three of these, or 20 per cent., gave negative results, or were possibly aborted very early. Three stillborn litters of nine individuals were produced. Sixty per cent. of the matings gave living litters, as against 44 per cent. in the first combination between treated males and normal females. The proportion of surviving young is, however, less from the treated females than from the treated males. Of 19 living young, 9 died soon after birth and 10 survived.

The third combination was between alcoholized males and females. Twenty-nine such matings gave in 15, or more than 50 per cent., of the cases negative results or early abortions. Three stillborn litters occurred, each consisting of two individuals. Only 11 living litters were produced containing 16 young, 9 of which survived while 7 died soon after birth.

All of the matings of the treated animals may be combined and compared with control matings as follows: In a total of 103 full term matings, 43, or almost 42 per cent., have given negative results or early abortions, while 35 control matings failed in only two cases, or about 6 per cent., to yield a full term litter. Fourteen, or 13½ per cent., of the matings gave stillborn litters consisting of

30 dead individuals. Only one stillborn litter occurred in the 35 control matings; this was a large litter of 4 individuals and the mother seemed almost unable to carry them. The 103 matings gave only 46 living litters, about 45 per cent., while 32 living litters, or $91\frac{1}{2}$ per cent., were produced by the 35 control matings.

The 46 living litters from the alcoholic matings contained 89 young, 37 of which died shortly after birth and 52 survived. The 32 living litters from the normal animals consisted of 60 individuals only 4 of which died while 56, or 93 per cent., of them survived.

Of 119 full term young, living and stillborn litters, produced by the alcoholic animals only 52, or less than 44 per cent., survived as against the 56, or $87\frac{1}{2}$ per cent., survivors among the 64 full term control offspring.

The offspring derived from the alcoholic individuals are termed second generation animals and were not themselves treated with alcohol. In three cases second generation individuals have been mated with normal and have given perfect results, although the litters have been small. It might seem as though the normal mate possessed a strong tendency to counteract any defect which may have been present in the second generation animal.

Mating second generation individuals with alcoholized guinea-pigs gave very different results. Two out of three such matings produced stillborn young, one of which was grossly deformed. The third mating gave two surviving young.

Nineteen matings have been made between second generation animals, the outcome of which compares very unfavorably with that from the control matings, while the data are closely similar to those obtained from the alcoholic matings. Seven, or almost 37 per cent., of the matings gave negative results. Twelve living litters were born consisting of 19 individuals, 6, or about 32 per cent., of which died very soon after birth and showed various nervous disorders; one was entirely eyeless and decidedly deformed.

From the number of records available one might conclude that the effects of the alcoholic treatment were as pronounced upon the offspring of the second generation animals, although they had not been directly treated, as upon the offspring of alcoholized individuals. The poison injures the cells and tissues of the body, the germ cells as well as other cells, and the offspring derived from the weakened or affected germ cells have all of the cells of their bodies defective, both soma and germ, since each of the cells is a descendant of the injured germ cell combination. In this manner the defects or degenerate conditions are transmitted or passed to subsequent generations.

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